(54) Title: ANTIBODIES AND THEIR USE IN CANCER THERAPY AND DIAGNOSIS

(57) Abstract

The present invention relates to antibodies, particularly to antibodies for use in cancer therapy and diagnosis, the antibodies having substantially the same binding specificity as EXT1-7C12 monoclonal antibody. This antibody is obtainable from a hybridoma cell line deposited at BCCM under reference LMBP1360CB. The antibody is shown to bind to a variety of carcinomas with a high intensity and degree of selectivity as demonstrated by immunohistochemistry analysis and cell binding assays. Compared to a panel of other monoclonal antibodies, the EXT1-7C12 antibody demonstrates a greater selectivity in binding to tumour cells, binding especially well to tumours of the ovary. The invention also includes antibodies having substantially the same binding specificity as monoclonal antibody EXT1-7C12, and mutants, derivatives, functional equivalents or fragments of the antibody. Applications of the antibodies include the therapy or diagnosis of cancer, e.g. in targeting cytotoxic compounds to cancer.

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ANTIBODIES AND THEIR USE IN CANCER THERAPY AND DIAGNOSIS

Field of the Invention

The present invention relates to the area of cancer therapy and diagnosis, and in particular to antibodies, compositions comprising the antibodies and the use of the antibodies in cancer therapy and diagnosis.

Background of the Invention

Monoclonal antibodies have a great potential in cancer therapy as they can bind to tumour antigens with great selectivity and guide cytotoxic activities to the cancerous cells. Thus, antibodies conjugated with cytotoxic compounds such as radioisotopes, drugs or toxins can be targeted to the tumour cells and inflict tissue damage on the tumours with varying degrees of selectivity (Goodman et al, 1993). Also naked antibodies can be used utilising natural defence mechanisms such as antibody dependent cellular cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC) against the tumour cells (Mujoo et al, 1987).

The degree of success in therapies using the antibodies depends on many factors, but an important factor is the restricted expression of the antigen recognised by the antibody to the tumour cells. Likewise, the extent of cross-reactivity of the antibody with other molecular structures and normal tissues is highly important. Thus, the ideal antibody would only bind to antigens expressed on tumour cells and not to any normal cells. Since most, if not all, tumour associated antigens are expressed both on normal as well as tumour cells (Sakamoto et al, 1986), the problem in the art has been to develop antibodies towards antigens expressed to a much higher degree on tumour cells than on normal cells.

The ability of any given monoclonal antibody to discriminate between tumour and normal tissue can to a large extent be determined by *in vitro* tests or animal model tests. Examination of the ability of the antibody to bind to tumour

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cells with selectivity can be performed with, for example, immuno-histochemistry, FACS analysis and other cell binding tests (Mittoti et al, 1987). Also the ability of the antibody to mediate cytotoxic effects can be studied *in vitro*

5 (Cheresh et al, 1985). Additional data concerning the suitability of the antibody to be used as a therapeutic or diagnostic drug can be gathered from animal model studies (Schreiber et al, 1992; Fujita et al, 1992; Isaacs et al, 1994).

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However, in many cases, only studies performed on patients will truly reveal the pertinent characteristics of the antibody. A large panel of monoclonal antibodies have been, and are presently, in various stages of clinical trials

15 (Labus et al, 1992; Kalofonus et al, 1989; Friedman et al, 1993; Goodman et al, 1993), but many of these monoclonal antibodies have failed to demonstrate significant clinical efficacy in extended trials. However, a recent report where a murine monoclonal antibody, 17-1A, which is directed on an

20 epitope on colo-rectal carcinomas, shows that this antibody is highly effective in prolonging the survival of patients over a five year period (Reitmuller et al, 1994).

Summary of the Invention

25 The applicants have developed a murine monoclonal antibody (EXT1-7C12) after PEG-induced fusion of murine splenocytes, immunised with human tumour cell lines, to HAT-sensitive SP2/0 myeloma cells. The antibody binds to a variety of with a high intensity and degree of selectivity as demonstrated by

30 immunohistochemistry analysis and cell binding assays. The antigen to which the antibody binds has not yet been fully identified, but is most probably a comparingly large membrane associated glyco-protein. The antigen is shed from the tumour cells and can be found in patients blood or in spent

35 culture medium from antigen expressing cells. Compared to an array of other monoclonal antibodies EXT1-7C12 demonstrates a more selective binding to tumour cells, binding especially

well to tumours of the ovary. The antibody has been demonstrated to target to human tumours transplanted into immunodeficient mice. Thus, the EXT1-7C12 monoclonal antibody shows unique features in binding activity and selectivity of human tumours and might prove useful for diagnosis and therapy of human tumours.

Accordingly, in one aspect the present invention provides antibodies having substantially the same binding specificity as monoclonal antibody EXT1-7C12 produced by the hybridoma cell line EXT1-7C12-B7-AA4-7A2-2C2 deposited at BCCM LMBP Culture Collection, Laboratory of Molecular Biology, University of Gent, K.L.Ledeganckstraat 35; B-9000 Gent, Belgium, the 14 March 1995, under reference LMBP1360CB.

In a further aspect, the present invention provides antibodies as obtainable from the hybridoma cell line deposited at BCCM under reference LMBP1360CB.

In a further aspect, the present invention provides a mutant, derivative, functional equivalent or fragment of antibody EXT1-7C12 cr an antibody having the binding specificity of the EXT1-7C12 antibody. Various methods for producing such modified antibodies are discussed at greater length below.

In a further aspect, the present invention provides the hybridoma cell line deposited at BCCM under reference LMBP1360CB.

In a further aspect, the present invention provides a pharmaceutical composition comprising one or more of the above antibodies, in combination with a pharmaceutically acceptable carrier.

In a further aspect, the present invention provides an antibody as described above for use in methods of medical treatment or diagnosis. In particular, the present invention

use of one or more of the above antibodies in the preparation
of medicaments for use in cancer therapy and diagnosis, and
more especially in the therapy or diagnosis of ovarian
cancer.

In a further aspect, the present invention provides a method
of determining the concentration or presence of cancer cells
using the above antibodies. One method involves associating
the antibodies with a label, exposing cells in a test sample
to the antibodies and determining the extent of binding by
detecting the label.

The specificity of the antibodies of the present invention
makes them particularly useful in targeting therapies to
cancer cells. As discussed above, these targeting therapies
are well known in the art and include so called ADEPT or
VDEPT techniques in which an active agent is administered as
a precursor, for conversion to an active form by an
activating agent targeted to or produced in the cells to be
treated. Thus, the activating agent can be targeted to the
cells by conjugation to antibodies of the present invention.

The antigen to which the EXT1-7C12 antibody binds has not yet
been fully identified but is most probably a membrane
integral glycoprotein. The unique binding profile of the
antibody also indicates that it has not been described
earlier. Compared to an array of other monoclonal antibodies
EXT1-7C12 demonstrates a more selective binding to tumour
cells. In particular, tumours of the ovary are intensely
stained.

The antibody has been demonstrated to target to human tumours
transplanted into immunodeficient mice. The EXT1-7C12
monoclonal antibody, thus, shows unique features in binding
activity and selectivity of human tumours, useful for the
diagnosis and therapy of human tumours.

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The EXT1-7C12 antibodies have a number of *in vivo* therapeutic applications. In addition to being used alone to target tumour cells, the antibodies may be used in conjunction with an appropriate therapeutic agent to treat human cancer. For example, the antibodies linked to therapeutic drug, such as cytostatica, cytokine or a radioisotope, for example ^{188}Rh or ^{90}Y or toxin, such as abrin or ricin for delivery of the therapeutic or toxic agent tumour cells according to well-known techniques.

The EXT1-7C12 may be administered *in vivo* or *ex vivo*, alone or in combination with one or more other antibodies.

The invention includes pharmaceutical compositions comprising a therapeutical effective amount of EXT1-7C12, either unmodified or a mutant, derivative, functional equivalent or fragment thereof, it may be conjugated or a part of an antibody cocktail for the purpose of treating cancer.

The antibody composition of the invention can be administered using conventional modes including intravenous, intraperitoneal, oral, intralymphatic or directly into the tumour. Preferably the antibody is administered parenterally.

The dosage forms may include liquid solutions, or suspensions, tablets, microcapsules, liposomes and injectable solutions. The precise dosage form will be dependent upon the mode of administration and the therapeutic application, and can be readily determined by the person of ordinary skill in the art.

The pharmaceutical compositions preferably include conventional pharmaceutically acceptable carriers and adjuvants known in the art such as human serum albumin, ion exchangers, alumina, lecithin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate and salts of electrolytes.

Modified Antibodies

The antibodies described above can be altered in a variety of
ways using recombinant DNA technology coupled with advances
in the field of monoclonal antibody and protein engineering.
This has enabled access to a large selection of antibodies
and antibody fragments with different properties and
structures to natural antibodies.

As discussed above, the production of monoclonal antibodies
is well established in the art. Monoclonal antibodies can be
subjected to the techniques of recombinant DNA technology to
produce other antibodies or chimeric molecules which retain
the specificity of the original antibody. Such techniques
may involve introducing DNA encoding the immunoglobulin
variable region, or the complementarity determining regions
(CDRs), of an antibody to the constant regions, or constant
regions plus framework regions, of a different
immunoglobulin. See, for instance, EP-A-184187, GB 2188638A
or EP-A-239400. A hybridoma producing a monoclonal antibody
may be subject to genetic mutation or other changes, which
may or may not alter the binding specificity of antibodies
produced.

As an alternative or supplement to immunising a mammal with
a peptide, an antibody specific for a protein may be obtained
from a recombinantly produced library of expressed
immunoglobulin variable domains, eg using lambda
bacteriophage or filamentous bacteriophage which display
functional immunoglobulin binding domains on their surfaces;
for instance see WO92/01047. The library may be naive, that
is constructed from sequences obtained from an organism which
has not been immunised with the target, or may be one
constructed using sequences obtained from an organism which
has been exposed to the antigen of interest (or a fragment
thereof).

Antibodies may be modified in a number of ways. Indeed the

term "antibody" should be construed as covering any specific binding substance having a binding domain with the required specificity. Thus this term covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including any polypeptide comprising an immunoglobulin binding domain, whether natural or synthetic. Chimaeric molecules comprising an immunoglobulin binding domain, or equivalent, fused to another polypeptide are therefore included. Cloning and expression of chimaeric antibodies are described in EP-A-0120694 and EP-A-0125023.

It has been shown that fragments of a whole antibody can perform the function of binding antigens. Examples of binding fragments are (i) the Fab fragment consisting of VL, VH, CL and CH1 domains; (ii) the Fd fragment consisting of the VH and CH1 domains; (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward, E.S. et al., Nature 341, 544-546 (1989)) which consists of a VH domain; (v) isolated CDR regions; (vi) F(ab')2 fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird et al, Science, 242, 423-426, 1988; Huston et al, PNAS USA, 85, 5879-5883, 1988); (viii) bispecific single chain Fv dimers (PCT/US92/09965) and (ix) "diabodies", multivalent or multispecific fragments constructed by gene fusion (WO94/13804; P. Holliger et al Proc. Natl. Acad. Sci. USA 90 6444-6448, 1993).

Diabodies are multimers of polypeptides, each polypeptide comprising a first domain comprising a binding region of an immunoglobulin light chain and a second domain comprising a binding region of an immunoglobulin heavy chain, the two domains being linked (eg by a peptide linker) but unable to associate with each other to form an antigen binding site: antigen binding sites are formed by the association of the

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first domain of one polypeptide within the multimer with the second domain of another polypeptide within the multimer (WO94/13804).

5   Where bispecific antibodies are to be used, these may be conventional bispecific antibodies, which can be manufactured in a variety of ways (Holliger, P. and Winter G. Current Opinion Biotechnol. 4, 446-449 (1993)), eg prepared chemically or from hybrid hybridomas, or may be any of the

10   bispecific antibody fragments mentioned above. It may be preferable to use scFv dimers or diabodies rather than whole antibodies. Diabodies and scFv can be constructed without an Fc region, using only variable domains, potentially reducing the effects of anti-idiotypic reaction. Other forms of

15   bispecific antibodies include the single chain "Janusins" described in Traunecker et al, Embo Journal, 10, 3655-3659, (1991).

Bispecific diabodies, as opposed to bispecific whole

20   antibodies, are also particularly useful because they can be readily constructed and expressed in E.coli. Diabodies (and many other polypeptides such as antibody fragments) of appropriate binding specificities can be readily selected using phage display (WO94/13804) from libraries. If one arm

25   of the diabody is to be kept constant, for instance, with a specificity directed against antigen X, then a library can be made where the other arm is varied and an antibody of appropriate specificity selected.

30   Previously, bispecific antibodies incorporating a specificity for the T-cell co-receptor CD3 have been shown to inhibit tumour growth (Titus, J. A. et al, J. Immunol. 138, 4018-4022 (1987)) and to cure lymphoma (Brissinck J. et al, J. Immunol. 174, 4019-4026 (1991)).

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It may be desirable to "humanise" non-human (eg murine) antibodies to provide antibodies having the antigen binding

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properties of the non-human antibody, while minimising the immunogenic response of the antibodies, eg when they are used in human therapy.   Thus, humanised antibodies comprise framework regions derived from human immunoglobulins

5   (acceptor antibody) in which residues from one or more complementary determining regions (CDR's) are replaced by residues from CDR's of a non-human species (donor antibody) such as mouse, rat or rabbit antibody having the desired properties, eg specificity, affinity or capacity. Humanised

10   antibodies may be prepared according to well known techniques in the art and are illustrated herein by the references Jones P.T., Dear P.H., Foote J., Neuberger M.S. and Winter G. "Preparing the complementary-determining regions in a human antibody with those from a mouse", Nature, 321: 522-525

15   (1986) and Verhoeyen M.E., Saunders J.A.,Price M.R., Marugg J.D., Briggs S., Broderick E.L., Eida S.J., Mooren A.T.A. and Badley R.A. "Construction of a Reshaped HMFG1 Antibody and Comparison of its Fine Specificity with That of the Parent Mouse Antibody", Immunology 1993, vol 78 pages 364-370. Some

20   of the framework residues of the human antibody may also be replaced by corresponding non-human residues, or by residues not present in either donor or acceptor antibodies.   These modifications are made to the further refine and optimise the properties of the antibody.

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The present invention will now be described by way of example with reference to the following drawings.

Brief Description of the Drawings

30   Figure 1 shows the cloning steps leading of the EXT1-7C12 antibody leading to the master working cell bank;

Figure 2 shows the staining of the Ovarian carcinoma cell line NIH:OVCAR-3 with EXT1-7C12

Figure 3 shows the binding of radiolabelled EXT1-7C12 to

35   NIH:OVCAR-3 cells

Figure 4 shows the uptake of radiolabelled EXT1-7C12 in different tissue types;

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Figure 5 shows the binding of EXT1-7C12 to purified antigen;

Figure 6 shows a western blott of a blott from a SDS-PAGE of shed antigen detected with EXT1-7C12 antibody;

Figure 7 shows the EXT1-7C12 antigen to be different from the MUC-2, MUC-3, MUC-4 and MUC-5 antigens;

Figure 8 shows the ELISA detection of EXT1-7C12 in the circulation of patients with ovarian cancer; and,

Figure 9 shows the lack of correlation between the ovarian cancer marker CA125 and EXT1-7C12.

Detailed Description

Production and characterisation of the hybridoma cell line and the EXT1-7C12 monoclonal antibody

Immune parental cell

Immune spleenocytes were obtained from Balb/c mice (Charles River UK. Ltd, Margate Kent). The mice which were negative for a number of pathogenic virus and bacteria were injected intraperitoneally (i.p.) with a mixture of proteins extracted from the human ovarian tumour cell line, NIH:OVCAR-3(Hamilton et al, 1983) in accordance with the following schedule.

Date	Amount of prep.	Adjuvant
920305	0.25 ml subcutaneously	0.25 ml FCA
920325	0.25 ml subcutaneously	0.25 ml FCA
920410	0.25 ml intra peritoneal	None
920602	0.25 ml intra peritoneal	None

FCA = Freunds Complete Adjuvant

NIH:OVCAR-3 is an ovarian carcinoma cell line. The cells were cultivated without addition of antibiotics in R10 medium, RPMI 1640 (Gibco, No 041-08170) with addition of L-glutamine (Gibco, No 043-05030), "non essential aminoacids" (Gibco, No 043-01140) and foetal calf serum (FCS, Gibco, No 011-06290). The cells were regularly checked for contaminating mycoplasma with the fluorescent dye Hoechst 33258 (Chen, 1972), an ultramicrochemical test (Uitendaal

et al., 1978) and with a filter hybridization technique using ^{32}P labelled restriction fragment H900 as a radioactive DNA-probe specific for mycoplasma (SVA) and were always found negative. The cells were kept in serial cultures and removed from the tissue culture flasks using a trypsin EDTA-solution (Gibco No 45300-019). The cells were washed in PBS before being inoculated into the mice. A solid NIH:OVCAR-3 ovarian carcinoma was established subcutanously in a SCID mouse (tested and found negative for virus and pathogens). The animal was transplanted with $1x10^6$ viable NIH:OVCAR-3 cells and the animal was killed when the tumour reached a size of 1 x 1 cm. The tumour was frozen and later thawed, minced with scissors and mixed with destilled water to make a final 20% w/v of tissue in water. The mixture was then run in a Omnimixer at speed setting 6 for 1 min. The preparation was admixed to an equal amount of 1.25 M perchloric acid and mixed with a magnetic stirrer at room temperature for 30 min. Insoluble material was finally spun down for 30 min at 27 000 g, and the supernatant was dialysed against water and PBS (PBS = 8 mM Na_2HPO_4, 2 mM KH_2PO_4,137 mM NaCl, 3mM KCl, pH 7.3)to create the tumour cell extract used for immunization.

A mouse was injected four times with 250 ul doses of the tumour celline extract. The last injection was a booster dose given three days before fusion.

Three days after booster inoculation of the mouse 120 $x10^6$ viable spleen cells were harvested and fused with $60x10^6$ SP2/0-Ag-14 myeloma cells using polyethylenglycol 1540 (Polyscience Inc, PA, No 1102). The SP2/0 myeloma cells were taken from a master working cell bank and the cells were kept in exponential growth phase and cultivated for less than 1 month prior to the fusion. The fusion mixture was distributed into Costar 96-well tissue culture plates at a cell density of 129 x $10^3/100\mu l$/well. Hybridomas were selected in DMEM-HAT medium (Gibco, No 041-01965 and No

043-01060) supplied with HECS (Human Endothelial Cell Supernatant, Costar, No C6110) used according to the manufacturers recommendation and with 10% foetal calf serum (Gibco, No 011-6290). The wells were supplied with fresh medium each week until supernatants from wells with growing hybrids were screened for binding activity against tumour cell lines utilising cell-ELISA techniques (se below).

Five different wells were classified as positive and were then cloned through limiting dilution. One of the wells was cloned four times seeding 1 cells/well for the first cloning and 0.1 cells/well for the last cloning step as illustrated in figure 1. The various steps were characterised by the following:

Fusion   920605

ELISA   920623

3 per cent of growing hybrids positive in cell-ELISA

Cloning 1 920703 1 cell/well

ELISA   920714

100 per cent of growing hybrids positive in cell-ELISA

Cloning 2 920728 0.2 cell/well

ELISA   920807

100 per cent of growing hybrids positive in cell-ELISA

Cloning 3 920904 0.1 cell/well

ELISA   920917

100 per cent of growing hybrids positive in cell-ELISA

Cloning 4 930930 0.1 cell/well

ELISA   931010

100 per cent of growing hybrids positive in cell-ELISA

This procedure resulted in the monoclonal hybridoma line EXT1-7C12-B7-AA4-7A2-2C2 and this cell line was expanded to Master Cell Bank (25 amp) and further to a Manufacturing working Cell Bank (50 amp) (figure 1). The monoclonality of the hybridoma cell line was ascertained by the fact that during the last two cloning steps all wells with growing hybridoma cells also produced antibodies binding to the

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antigen.

Further analysis including PAGE, isoelectric focusing, FPLC and determination of isotype have all confirmed this conclusion. The cloning procedure was performed in HECS-supplemented medium without addition of feeder cells.

The EXT1-7C12-B7-AA4-7A2-2C2 monoclonal hybridoma cell line produces a monoclonal antibody called EXT1-7C12 which is a murine IgG$_1$ kappa antibody as determined by immunodiffusion and ELISA assays using isotype-specific (ICN Biomedicals Inc, USA) and light chain specific (Southern Biotechnology Associated Inc, USA) antibodies respectively. The hybridoma was found to produce 5.2 μg antibody/ml/10^6 cells/24h under exponential growth in a tissue culture flask. No production of immunoglobulin originating from the myeloma cell line could be found, and analysis of the sequence of parental mRNA coding for Ig-chains revealed the inclusion of a stop codons and modifications in the Ig-genes obliterating the translation of mRNA into protein.

Activity of the antibody

Binding to tumour cell lines as analysed with ELISA

Monolayers of tumour cells in 96 well tissue culture plates were dried at 37°C over night and fixed with 0.025% glutaraldehyde in PBS for 10 min. After fixation the plates were washed in PBS and residual glutaraldehyde was inactivated with 50% FCS in 0.1M glycine, 50 mM TRIS-HCl buffer pH 7.8. The wells were washed with 0.01 M sodium phosphate buffer pH 7.3, containing 0.15 M sodium chloride and 0.05% Tween 20. Culture supernatants to be tested for presence of anti-tumour antibodies were serially diluted, added to the wells containing tumour cells and incubated for one hour at 37°C. Bound antibody was then detected with horseradish peroxidase (HRPO) conjugated to a rabbit anti-murine Ig (P260, Dako A/S, Glostrup, Denmark) for one

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hour. After additional washing steps 200 μl of developing
solution (3.7 mM ortho-phenyldiamine in 0.05 M citric acid,
pH 5.0) was added to each well. The reaction was stopped
after 10 min by adding 50 μl of 1M H_2SO_4 to each well, and
5   the absorbance was read at 492 nm in a Multiscan MCC/340.
Absorbance three times above the media background was
regarded as positive (+). A summary of the results is
presented in Table 1.

10   <u>Binding to tumour cell lines as analysed by flow cytometry</u>
The tumour cell lines were tested for reactivity with
hybridoma supernatants using an indirect immunofluoresence
assay. Adherent cell lines were treated with trypsin and
washed in PBS containing 1% bovine serum albumin (PBS-BSA)
15   while non adherent cells were incubated directly with
antibody containing culture supernatant. The cells (0.5-
$1x10^6$) were incubated with 100 μl diluted hybridoma cell
culture supernatant for 45 min on ice, washed as above, and
reincubated for 45 min on ice with a FITC conjugated goat
20   anti-murine Ig (Dako A/S). The cells were then washed and
analysed on a FACStar plus flow cytometer (Becton
Dickinson, San José, CA) as illustrated in Figure 2. The
white signal represents the EXT1-7C12 stained NIHOVCAR-3
cells and the black signal represents the background signal
25   from cells stained with the negative control. A summary of
the results is presented in Table 2. A variety of tumour
cell lines with hematopoietic origin were tested, but none
of them were stained with the EXT1-7C12 antibody.

30   Differences were found between results obtained from the
ELISA-tests and flow cytometric analysis as, for example
PC-3 was stained when analysed in ELISA, but was found
negative in flow cytomeric tests. Possibly the trypsin
used in the flow cytometry tests reduced the amount of
35   antigen on the cell surface.

<u>Immunohistochemistry</u>

Tissue specimens used for immunostaining were removed during surgery or autopsy and snap frozen in isopentane within one hour after removal. The tissues were cut into 5μm thick sections using a cryotome. The sections were dried over night at room temperature and fixed in acetone at -20°C for 10 min. After fixation, the sections were soaked for 30 min at +20°C in TRIS buffer containing 10% FCS and then incubated at room temperature for 1 hour with antibody containing supernatants, diluted to give a final Ig-concentration of 5-10 μg/ml. The sections were washed 3 times and biotin labelled goat anti-murine Ig was added. After incubation for 30 min a horeseradish peroxidase avidine biotin complex (VECTASTAIN ABC-kit, Vector Laboratories Inc., Burlingame, CA) was added and incubated for 30 min. The sections were washed and supplied with a solution containing diaminobenzidine (DAB) to visualize bound antibody. After a final washing step, the sections were dried and mounted in mounting media (PERTEX, Histolab, Sweden) before examined in a microscope. Tumours were considered positive if more than 10% of the tumour cells were clearly stained. In many tumours, all neoplastic cells were found to express the antigen. The staining was associated with the plasma membrane of tumour cells and gave an overall impression of a mucin type of staining. A summary of the staining results is shown in Table 3. The EXT1-7C12 antibody bound a large portion of human ovarian carcinomas, but also to breast and lung carcinomas. The EXT1-7C12 also bound to some normal tissue, although to a low frequency and with a low intensity.

The EXT1-7C12 also bound to formalin fixed and paraffin embedded tissue. When a number of routine pathological ovarian tumour specimens were tested for EXT1-7C12 binding it was found that most of the tumours were positively stained (Table 4).

The results from this investigation revealed that the EXT1-

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7C12 antibody bound a high frequency of ovarian carcinomas. It is also clear from table 4 that EXT1-7C12 binds the majority of the serous cystadenomas, the most frequent type of ovarian tumour. Since it is well known that most, if not all, monoclonal antibodies to tumour associated antigens also bind to normal tissue to varying degrees, the staining pattern of the EXT1-7C12 antibody was compared to that of four other murine monoclonal antibodies already used in clinical trials for immunolocalization and therapy of tumours (Table 5).

The 17-1A (Reitmÿller et al, 1994) and KS 1/4 (Labus et al, 1992) monoclonal antibodies which probably recognize different epitopes on a tumour associated antigen present on many different carcinomas (Strnad et al, 1989) together with HMFG-1 (Epenetos et al, 1987, Kalofonos et al, 1989, Hammersmith Oncology group 1984), which was raised against a human mucin (Taylor-Papadimitriou et al, 1981) present in many different types of adeno carcinomas (Girling et al, 1989) and the B72.3 antibody (Esteban et al, 1987) recognizing a sialoyl containing carbohydrate antigen enriched on many different forms of tumours (Ohuchi et al, 1986; Xu et al, 1990; Cordoncardo et al, 1992; Bryne et al, 1990; Inoue et al, 1991; Johnston et al, 1986) were evaluated. A nonbinding IgG_1 antibody and cell culture medium were used as controls.

When compared with the four monoclonal antibodies described above the EXT1-7C12 was found to be as broad in its tumour interaction as they were, but had much less cross reactivity with normal tissue (Table 5).

The 17-1A and KS1/4 antibodies showed a more homogeneous binding to the ovarian tumours tested. However, their binding to normal tissue was also very pronounced. Besides binding to epithelial cells in kidney, colon, stomach, pancreas, oesophagus and breast the KS1/4 also bound to

peripheral nerve and to endothelium in small blood vessels in brain and placenta. In the liver, both the bile collecting ducts and some arteries were stained. The intensity of the staining was as strong in normal tissue as in tumour tissue. 17-1A showed a similar pattern, but stained weaker than KS1/4. Staining with both HMFG-1 and B72.3 was associated with mucin producing tissue and stained the apical parts of tumour as well as normal cells. Both antibodies also stained kidney tubule, normal epithelia in ducts of the breast and the ascini of pancreas. HMFG-1 stained all ovarian carcinomas but the intensity of the staining was similar in normal colon and tumour tissue. In our hands, the HMFG-1 antibody stained all normal striated muscle tested with a low intensity. The B72.3 antibody also stains cell nucleus and some normal blood vessels in the brain, this staining was weak but reproducible and found in most brain tissue tested. A staining of blood vessels in the brain was also observed with KS1/4.

In conclusion, the EXT1-7C12 showed both less frequent and less intense cross reactivity with normal tissue as compared to the other monclonal antibodies.

It is foreseeable that the EXT1-7C12 antibody, if given to patients, could reach quite high concentrations in plasma and in tissue. To study whether such high concentrations could lead to unspecific binding to normal tissue various concentrations of the EXT1-7C12 antibody, and for comparison the KS1/4 antibody, were titrated from 100 to 0.0001 μg/ml on human tissue sections and evaluated by immunohistochemistry (Table 6). Estimating the plasma volume in a patient to be around 5 liters the highest dose used here in IHC (100 μg/ml) would correspond to a dose given to patients in excess of 500 mg.

The binding of EXT1-7C12 to tumour was more intense than

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that of the KS1/4 antibody. The EXT1-7C12 still gave a clearly detectable staining on transplanted NIH:OVCAR-3 even when incubated at such low concentration as 0.01 mg while the KS1/4 antibody was found negative at concentrations below 0.3 mg/ml. The staining of normal tissue was however less intense. The EXT1-7C12 antibody only faintly stained normal tissue and only when used at the highest concentration.

This indicates that the antigen recognised by the EXT1-7C12 antibody was absent or only present in a very low concentration in normal tissue. The results also suggest that the EXT1-7C12 antibody, when administered to patients in doses less than 5 mg, will not bind unspecifically to tissues not expressing the antigen.

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In vivo activity of the EXT1-7C12 antibody

Ability of the EXT1-7C12 antibody to localise to human tumours transplanted to immunodeficient SCID mice
5 ### Localisation of unlabelled antibody

SCID mice which had been tested and found to have a serum-concentration of murine antibodies less than 1 mg/ml were used in these studies. The mice were inoculated subcutaneously with 0.1 ml cell suspensions containing 1 -
10 2 x 10^6 viable tumour cells in each shoulder. Each animal was supplied with one EXT1-7C12 antigen negative (M24 met) and one EXT1-7C12 antigen positive tumour (NIH:OVCAR-3). The tumours were allowed to grow to the size of approximately 8x8 mm when the animals were injected i.p.
15 with 250 mg of purified EXT1-7C12 antibody dissolved in sterile PBS. The animals were sacrificed 1, 2 or 3 days after injection of antibodies and both tumours as well as liver and kidney were frozen and analysed in immunohistochemistry for the presence of antibody.

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The experiment showed that antibody localised well and immunoglobulin could be found in the antigen expressing NIH:OVCAR-3 tumour from day 1 onwards, whereas the M24 met tumour did not contain any detectable antibody. Less but
25 still significant amounts of antibody were also found in the liver and to some extent in the kidneys. The presence of minor amounts of antibody in liver and kidney might be explained by the fact that levels of antibody was found in the circulation of the animals throughout the experimental
30 period.

Localisation of radiolabelled antibody to transplanted tumours

Purified EXT1-7C12 antibody were labelled with ^{125}I using
35 the ATE method (Schuster et al, 1991). In brief, 50 μl 1% acetic acid in methanol, 25 μl N-succininimidyl-3-(tris-n-butylstannyl)benzoate (ATE) 1mg/ml, 12 μl of N-

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chlorosuccinimide 5mg/ml and 30 μl Na I^{125} (114 MBq, Amersham, IMS 30) was mixed in a glas tube and incubated for 15 min. The reaction was terminated by the addition of 10μl NaS$_2$O$_3$ 5mg/ml and stirred for another 5 min. The mixture was evaporated to dryness under nitrogen.

72 μl of EXT1-7C12 (7 mg/ml) in 0.2 M borate buffer, pH 8,7 were added to the tube, mixed and incubated at room temperature for 10 min. Then 300 ml of 20 mg/ml glycine in borate buffer pH 8,7 was used to terminate the labelling reaction under an additional 5 min. The labelling efficiency were checked with TLC (thin layer chromatography). Labelling efficiency (the amount of added iodine adhering to the protein) was estimated to 56% and the specific activity of the antibody was determined to be 63MBq/500 mg.

The radiolabelled antibody was then separated from free iodine through gelfiltration on a Pharmacia PD-10 columns equilibrated with 0.1 M Phosphate buffer, pH 7.2 and pretreated with 1% solution of BSA.

To evaluate the affinity constant of the EXT1-7C12 antibody labelled with ^{125}I was incubated with NIH:OVCAR-3 cells. Briefly, 1x10^6 NIH:OVCAR-3 cells and 100 ml of different amounts of EXT1-7C12 antibody were mixed in tubes and incubated on ice for 60 min.

The mixture was then centrifuged through 200 ml of 40% of Ficoll to separate bound antibody from free. Radioactivity in supernatant (free) and cell pellet (bound) was then determined in a gamma counter (Table 7).

Samples of 50 ml of the supernatants were removed and analysed in a gamma counter for determination of the amount of unbound antibody. The tube were then frozen, the cell pellet containg the bound antibody were cut off and

analysed in a gamma counter giving the amount of bound radioactivity (Table 7).

The data presented in table 7 was then analysed in a Scatchard plot (Figure 3). When the amount of cell bound antibody (B) was plotted against cell bound antibody/free antibody (B/F) the affinity constant could be determined to be 0.9×10^9 M^{-1} and the number of epitopes per cell on NIH:OVCAR-3 cells to be 230 000 antibody epitopes/cell (Figure 3).

The ability of radiolabelled EXT1-7C12 antibody to target selectively to antigen-expressing tumours NIH:OVCAR-3 and not to M24 met tumours not expressing the antigen was studied in SCID mice. The tumours were established on the hinds of SCID mice as described above. The tumours were allowed to reach an average size of 8 x 8 mm. A total amount of 10 mg of EXT1-7C12 antibody with a total activity of 1.3 MBq was injected in the tail vein of each mouse. The animals were observed with a gamma camera 6, 24, 48, 72, 96 and 124 hours after injection of the antibody. Localisation of antibody to the antigen positive tumour NIH:OVCAR-3 on the right side of the animal could be observed whereas no accumulation of radioactivity was found to the antigen negative tumour (Figure 4). However, a large fraction of the radiolabel was found in the abdomen and thorax of the mice. Since the thyroid gland had not been blocked by an excess of free iodine, a successfully increasing amount of radioactivity was also found to the area of the thyroid gland. A time dependent increase in the percentage of total radioactivity localised to the antigen expressing tumour was also indicated. To obtain more accurate measurements of the distribution of the radiolabel five animals were sacrificed on each time point and different organs were removed and analysed for radioactivity in a gamma counter. The percentage of injected dose/g was calculated and the data are presented

in figure 4a. The antibody was found to localise with preference to the antigen expressing tumours. Radioactivity was not found to accumulate in any tissue exept the NIH:OVCAR-3 tumour and the thyroid gland (figure
5   4b).

Characterisation of the antigen

The exact nature of the antigen and the specific epitope identified by the EXT1-7C12 antibody have not yet been
10   fully characterised. However, several findings suggest the antigen to be a protein associated with the plasma membrane of tumour cells. Moreover, the antigen is shed and could be purified from the culture supernatant or from plasmamembrane. The surfaces of viable NIH:OVCAR-3 cells
15   were labelled with ^{125}I using the jodogen method (Fraker et al, 1978) the cells were lysed in 0.5 % Triton X-100 and the antigen precipitated with EXT1-7C12 antibody and protein A conjugated to sepharose. The antibody precipitated a large molecule that enters into the very
20   upper part of a 8 % SDS-PAGE gel, corresponding to a protein larger than 1×10^6 Da in molecular weight.

EXT1-7C12 antibody conjugated to Sepharose was used for affinity purification of the antigen found in the
25   supernatant from cultivated NIH:OVCAR-3 cells. The affinity-column was eluted with 2 M guanidinium chloride and the collected antigen was dialysed against PBS buffer and used to coat 96 well microtiter plates. In ELISA analysis, the eluate gave high intensity signal with the
30   EXT1-7C12 antibody, while other antibodies specific for large mucin molecules and with similar reactivity in immunohistochemistry (HMFG-1, HMFG-2 and SM-3) were negative (Figure 5a). In addition, the EXT1-7C12 did not bind to a synthetic peptide containing the 20 aminoacids
35   (TAPPAHGVTSAPDTRPAPGS) from the tandom repeat of the MUC-1 mucin recognised by the monoclonal antibodies HMFG-1, HMFG-2 and SM-3 (Figure 5b).

The affinity purified antigen was separated on a gradient 4-20% SDS-PAGE gel and blotted to a nitrocellulose paper and analysed with Western blotting technique. Increasing

5   amounts of antigen was separated on two identical gels and blotted to nitrocellulose paper. Blot a) was incubated with EXT1-7C12 and blot b) was incubated with a control antibody. Peroxidase conjugated rabbit anti-mouse antibody was used as a secondary step and binding was revealed with

10   chemiluminisens (Figure 6). Shed antigen was similar to plasma membrane bound antigen in its migration in SDS-PAGE (data not shown).

The epitope seems to be of peptide nature since it was

15   insensitive to treatment with endo or exoglycanases. Treatment with neuraminidase, β-galactosidase, Endo-F/peptide-N-glycosidase F or Endo a-N-acetylgalactosaminidase did not alter the binding of the antibody when tested in ELISA (data not shown). Neither

20   did oxidative treatment with 10 mM sodium periodate or mercaptoethanol change the binding of the antibody (Figure 5a). Treatment with proteases did however effect the binding. Overnight treatment with high concentration of thermolysin did lower the binding of the EXT1-7C12 antibody

25   in ELISA.

According to the results with the HMFG-1, HMFG-2 and SM-3 antibodies the antigen is different from MUC-1 (figure 5). In order to examine if the affinity purified antigen is

30   associated with any other known mucine, the antigen preparation was dotted to a nitrocellulose membrane and treated with hydrofloride (HF) in order to remove all carbohydrate side chains without disrupting the apoprotein (Mort et al, 1977). The purified EXT1-7C12 antigen was

35   diluted and placed into small bands on six different nylon membrane. The amount of antigen applied to the membrane was from the top 40 mg, 8 mg, 1.6 mg, 0.32 mg, 64

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ng, 13 ng, 3 ng, 0.5 ng and 0.1 ng. Membrane 1 was left
untreated, while the rest of the membranes were treated
with HF to remove all carbohydrates.

5   Membrane 1 and 2 were incubated with EXT1-7C12 and the
other membranes were incubated with different polyclonal
antisera specific to MUC-2 (3), MUC-3 (4), MUC-4 (5) and
MUC-5 (6). Bound antibody were detected with a secondary
antibody conjugated with alkaline phosphate and the bands
10   were visualised with NBT (Figure 7).

The antigen turned out to be negative to al of the
reagenses and no specific binding could be detected in the
HF treated antigen. Only the higest concentration of
15   antigen gave a weak background staining of the antigen.
The antigen was also found to lose its EXT1-7C12 epitope
after HF treatment.

Since the antigen is shed from cultivated tumour cell
20   lines, the antibodies disclosed herein can be used to
detect the antigen circulating in tumour patients. To
verify this, we performed an analysis using a sandwich
assay consisting of EXT1-7C12 as both catching and
detecting antibody. 100ml of 2 mg/ml of EXT1-7C12 antibody
25   was coated to the wells of a 96 well plate for 1 h in 37°C.
The wells were washed with PBS 0,05% Tween 20 (0.01 M
sodium phosphate buffer pH 7.3, containing 0.15 M sodium
chloride and 0.05% Tween 20). Sera from tumour patients
and healthy blood donors were incubated for 45 min diluted
30   1/5 in PBS 0.05% Tween 20. After washing with the same
buffer the wells were incubated with biotin labelled EXT1-
7C12. Bound antibody was then detected with horseradish
peroxidase (HRPO) conjugated to streptavidin(Dako A/S,
Glostrup, Denmark) for 45 min. After additional washing
35   steps, 200 µl of developing solution (3.7 mM ortho-
phenyldiamine in 0.05 M citric acid, pH 5.0) was added to
each well. The reaction was stopped after 10 min by adding

50 μl of 1M H_2SO_4 to each well, and the absorbance was read
at 492 nm in a Multiscan MCC/340. EXT1-7C12 units were
calculated from 100 x OD (492). In figure 8, the results
from patients with advanced decease are compared with the
results from blood donors. The results from the tumour
patients were also compared to the CA125 values (Abbot
diagnostic) from the same sera (Figure 9).

Conclusions of preclinical evaluation

The EXT1-7C12 antibody differs from other antibodies
recognising tumour associated antigens on ovarian
carcinomas. The best documented anti-carcinoma antibodies
with specificities for ovarian cancer are: HH8 (anti-Galb
(1-3) GalNac-a-O-Ser/Thr) (Kjeldsen et al, 1974), MLS-128
(anti Tna = GalNac-a-O- Ser/Thr) (Nakada et al, 1992), anti
CEA (Hammarstrśm et al,1989), B72.3 (Johnsson et al, 1986),
CA125 (Bast et al, 1987), KS-1/4 (Bumol et al, 1988) and BR
96 (anti-Le Y, Hellstrśm et al,1990) stain differently in
IHC and flow cytometry. The EXT1-7C12 antibody stains
ovarian carcinomas to an equal or better intensity than
other antibodies as judged from the literature and in
direct comparing experiments on frozen tissue sections. In
a serum assay for circulating antigen, the presence of
EXT1-7C12 does not correlate with the most frequently used
serum marker CA 125.

The antigen recognised by EXT1-7C12 seems to be a large
plasma membrane associated glycoprotein differing from the
well characterised MUC-1,2,3,4 and 5 mucins. EXT1-7C12
gives a much lower normal tissue binding compared to other
anti-mucin antibodies. Taken together with the fact that
both radiolabelled and unconjugated EXT1-7C12 antibody
localised to the NIH:OVCAR-3 explanted tumours, but not to
antigen negative melanoma in SCID mice, EXT1-7C12 looks
like a promising antibody for diagnostic and therapeutic
use in patients suffering from ovarian carcinoma.

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Table 1

Ability of EXT1-7C12 to bind to cell lines

Cell line	Tumour origin	Binding
COLO 205	Colon cancer	-
PANC-1	Pancreatic cancer	-
BxPC		-
MIA PaCa-2		-
ASPC-1		-
Caov-3	Ovarian cancer	+
SK-OV-3		-
NIH:OVCAR-3		+
MCF7	Breast cancer	-
T-47		-
PC3	Bladder cancer	+
PA1	Teratoma	-
HEL8	Embryonal Lung Fibroblasts	-
A 549	Lung cancer	+
Jurkat	Leukaemia (ALL)	-

Different cell lines were stained with the EXT1-
7C12 antibody in an ELISA test. Bound antibody was
detected with HRPO-labelled rabbit anti-murine Ig.
Wells with a light absorbance three times above
the background were regarded as positive (+)

Table 2

Ability of the EXT1-7C12 antibody to bind to
cell lines as analysed by flow cytometry

Cell line	Tumour origin	Per cent positive cells
COLO 205	Colon cancer	1
PANC-1	Pancreatic tumour	5
MIA PaCa-2		31
ASPC-1		5
BxPc		2
Caov-3	Ovarian cancer	13
SK-OV-3		4
NIH:OVCAR-3		99
T-47	Breast cancer	3
PC3	Bladder cancer	3
PA1	Teratoma	2
HEL8	Embryonal Lung Fibroblasts	2
A 549	Lung cancer	30

The cells were stained in suspension using a
FITC-labelled secondary anti-mouse Ig
antibody.

Table 3

Immunohistochemical analysis of binding of the EXT1-7C12 antibody to tissue sections.

Tissue	# positive/ # tested	Positive cells
Normal		
Brain	0/8	
Peripheral nerve	0/1	
Lung	0/9	
Liver	0/10	
Kidney	0/12	
Ovary	0/7	
Mammary gland	3/4	A few cells in ductal epithelium
Oesophagus	0/1	
Stomach	5/11	A faint staining of some mucin in the upper mucosa
Colon	10/15	A faint staining of some epithelial cells in crypts
Tonsil	0/13	
Lymphnode	0/8	
Pancreas	0/4	
Muscle	0/12	
Peyers plaque /colon	0/10	
Placenta	0/4	
Tumour		
Colo/rectal	2/14	
Ovarian	10/14	
Lung	5/15	
Pancreatic	1/8	
Breast	5/10	

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Table 4

Immunohistochemical analysis of binding of the
EXT1-7C12 antibody to paraffin embedded tissue
sections of different ovarian tumours.

Class of ovarian tumour	Degree of differentiation	EXT1-7C12 binding
Cystadenomas, serous	Low	++
	Low	-
	Low	++
	Medium	+
	Medium	++
	Medium	+
	Medium	+
	High	+
	High	++
	High	+
Cystadenom, mucinous		-
Cystadenom, mucinous		-
Cystadenom, mucinous		+/-
Cystadenom, mucinous		-
Cystadenom, mucinous		-
Borderline tumour		++
Endometroid tumour		+++
Endometroid tumour		+/-
Endometroid tumour		-

Table 5

Immunohistochemical analysis of four monoclonal antibodies used in clinical trials.

Normal tissue	17-1A	KS1/4	HMFG-1	B72.3
Brain	0/6	6/6 Staining of blood vessels	0/6 Brain	6/7 Staining of cell nuclei and some blood vessels
Peripheral nerve	0/1	1/1 Nerve fibre	0/1	0/1
Lung	0/7	7/7 Columnar epithelium	7/7 Mucin	0/7
Liver	0/7	7/7 Bile ducts	0/7	0/7
Kidney	4/4 some tubules	4/4 Some tubules	4/4 Some tubules	3/4 Few tubules
Ovary	0/5	5/5 Connective tissue	0/5	0/5
Mammary gland	0/3	3/3 Cells in ductal epithelium	3/3 Mucins associated with cells in ductal epithelium	3/3 Some spots of mucins associated with cells in ductal epithelium
Oesophagus	1/1 epithelia	1/1 Epithelia	1/1 Mucin associated	1/1 Mucin associated
Stomach	8/8 Surface epithelium of mucosa. Parietal cells and cells in gastric glands	8/8 Surface epithelium of mucosa. Parietal cells and cells in gastric glands	8/8 Mucin of mucosa, heterogeneous staining of gastric glands	3/8 Mucin of mucosa
Colon	10/10 Epithelial cells in mucosa	10/10 All epithelial cells in mucosa.	10/10 Mucin and weak staining of all cells in crypts of mucosa. Muscle and muscularis mucosae.	10/10 Mucin associated

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Table 5   (continued)

Normal tissue	17-1A	KS1/4	HMFG-1	B72.3
Tonsil	0/11	10/11 Squamous epithelial cells	10/10 Outer portion on some stratified squamous epithelial cells	10/10 Staining of the most upper layer of some mucosa
Lymphnode	0/3	1/3 Epithelial cells	0/3	0/3
Pancreas	5/6 Some acinar cells	5/6 Acinar cells	5/6 Mucin in acinar cells	4/6 Weak staining of some acinar cells
Muscle	0/10	10/10 Patched staining in different types of muscle	10/10 Diffuse staining of all muscles	0/10
Peyers plaque / colon	0/10	0/10	0/10	0/10
Placenta	1/4 Blood vessels in foetal part	4/4 Blood vessels in foetal part	3/4 Outer portion of some syncytio-trophoblasts	0/4
Tumour tissue				
Colo/rectal	15/15 Intense homogeneous staining	15/15 Intense homogeneous staining	12/15 Apical staining of cells but not stronger than to normal colon	13/15 Heterogeneous apical staining
Ovarian	8/9	9/9	4/4	6/11
Lung	20/22	6/22	ND	3/3
Pancreatic	3/3	3/3	3/3	3/3
Breast	3/6	7/7	7/7	3/6

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Table 6

Binding of EXT1-7C12 and KS 1/4 monoclonal antibodies to tumour and normal tissue as assessed by immunohistochemistry

µg/ml	NIH:OVCAR		A549		Kidney		Tonsilla		Ovarium		Lung	
	EXT1-7C12	KS 1/4	EXT1-7C12	KS 1/4	EXT1-7C12	KS 1/4	EXT1-7C12	KS 1/4	EXT1-7C12	KS 1/4	EXT1-7C12	KS 1/4
100	+++	++	++	++	(+)	++	(+)	-	-	+	+	++
10	+++	++	-	++	-	++	-	-	-	-	-	+
5	++	++	++	++	-	++	-	÷	-	-	-	÷
2.5	++	++	++	++	-	++	-	-	-	-	-	+
1.25	++	++	++	++	-	++	-	÷	-	-	-	(+)
0.6	++	÷	++	++	-	+	-	-	-	-	-	(+)
0.3	++	÷	++	÷	-	÷	-	-	-	-	-	(+)
0.15	++	(-)	++	-	-	(+)	-	-	-	-	-	(+)
0.07	+	-	÷	(-)	-	(+)	-	-	-	-	-	-
0.01	+	-	-	-	-	-	-	-	-	-	-	-
0.001	(+)	-	-	-	-	-	-	-	-	-	-	-
0.0001	-	-	-	-	-	-	-	-	-	-	-	-
0	-	-	-	-	-	-	-	-	-	-	-	-

- to +++ designates subjective evaluation of the staining intensity

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Table 7

Ability of radio labelled EXT1-7C12 antibody to
bind to NIH:OVCAR-3

125 I labelled 7C12 ng	CPM bound B	CPM free F	(ng bound) B
100	167167	143301	54
50	112323	54834	36
25	43377	22301	14
12,5	20612	9640	6,8
6,25	14947	4748	5,0
3,12	8375	2164	2,9
0,78	954	231	0,5

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A. The indications made below relate to the microorganism referred to in the description on page **3**, line **7 to 14**	

B. IDENTIFICATION OF DEPOSIT   Further deposits are identified on an additional sheet ☐

Name of depositary institution

    BELGIAN COORDINATED COLLECTION OF MICROORGANISMS (BCCM)

Address of depositary institution *(including postal code and country)*
    LABORATORY OF MOLECULAR BIOLOGY
    UNIVERSITY OF GENT
    K.L. LEDEGANCKSTRAAT 35
    B-9000 GENT
    BELGIUM

Date of deposit	Accession Number
14 MARCH 1995	**LMBP 1360 CB**

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on which such applications are refused or deemed to be withdrawn, a
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CLAIMS:

1. An antibody having the substantially the same binding specificity as monoclonal antibody EXT1-7C12 produced by the hybridoma cell line deposited at BCCM under reference LMBP1360CB.

2. An antibody as obtainable from the hybridoma cell line deposited at BCCM under reference LMBP1360CB.

3. A mutant, derivative, functional equivalent or fragment of an antibody according to claim 1 or claim 2.

4. An antibody according to claim 3 wherein the antibody is humanised.

5. Hybridoma cell line deposited at BCCM under reference LMBP1360CB.

6. A pharmaceutical composition comprising an antibody according to any one of claims 1 to 4, in combination with a pharmaceutically acceptable carrier.

7. The antibodies according to any one of claims 1 to 4 for use in a method of medical treatment or diagnosis.

8. The antibodies according to claim 7 for use in cancer therapy or diagnosis.

9. The antibodies according to claim 8 wherein the cancer is ovarian cancer.

10. The antibodies according to any one of claims 7 to 9 wherein the antibodies are for use in targeting cancer cells.

11. The antibodies according to claim 10 wherein the

antibodies are conjugated to a cytotoxic compound or a label, the antibodies being capable of selectively binding to the cancer cells so that the cancer cells can be killed or detected.

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12. Antibodies according to claim 11 wherein the cytotoxic compound is a radioisotope, a drug or a toxin.

13. The use of the antibodies of any one of claims 1 to

10   4 in the preparation of a medicament for the treatment or diagnosis of cancer.

14. A method of determining the concentration or presence of cancer cells in vitro using the antibodies of

15   any one of claims 1 to 4, the antibodies being associated with a label, the method comprising exposing cells in a test sample to the antibodies and determining the extent of binding by detecting the label.

20

EXT1-7C12

$\downarrow$

B7

$\downarrow$

AA4

$\downarrow$

7A2

$\downarrow$

2C2

Figure 1
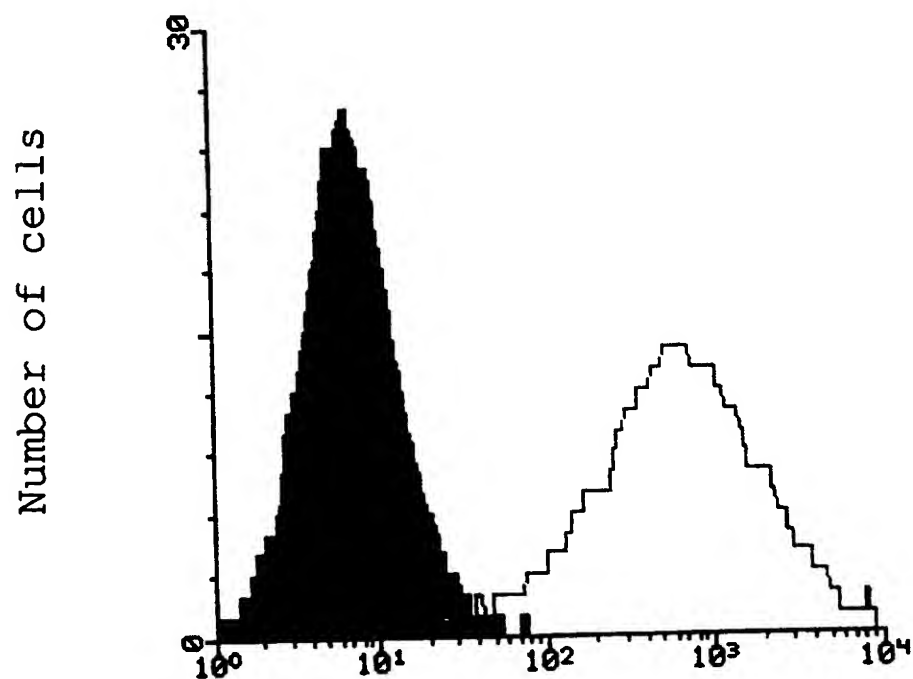
Log fluoresence intensity

Figure 2
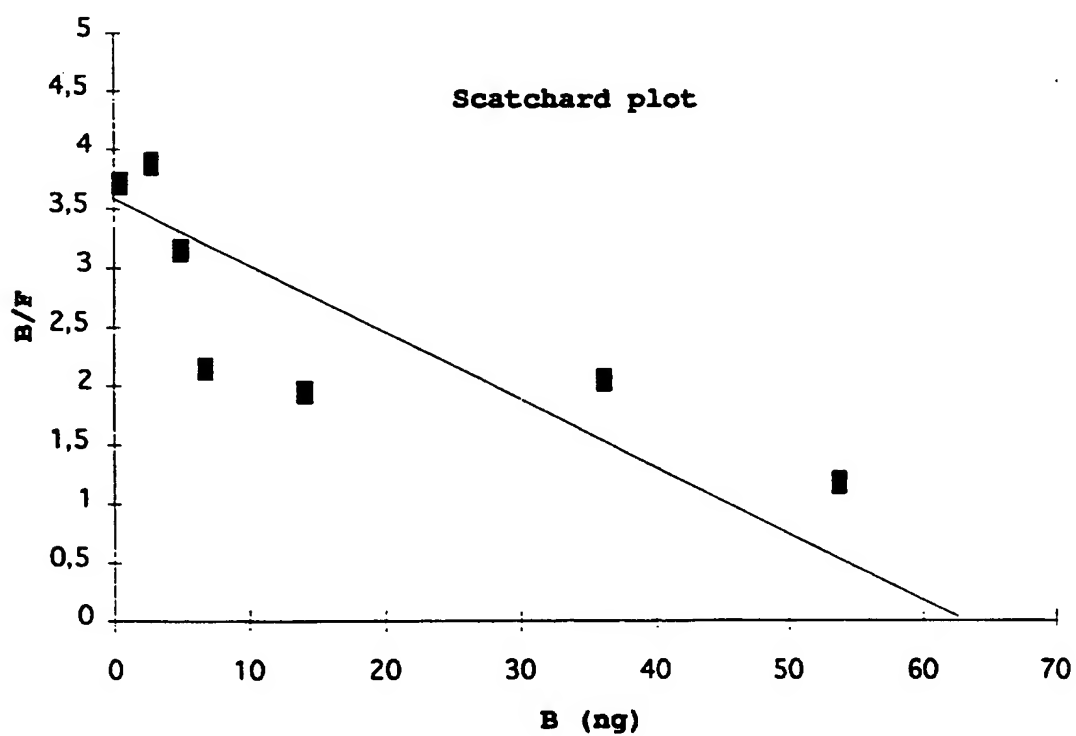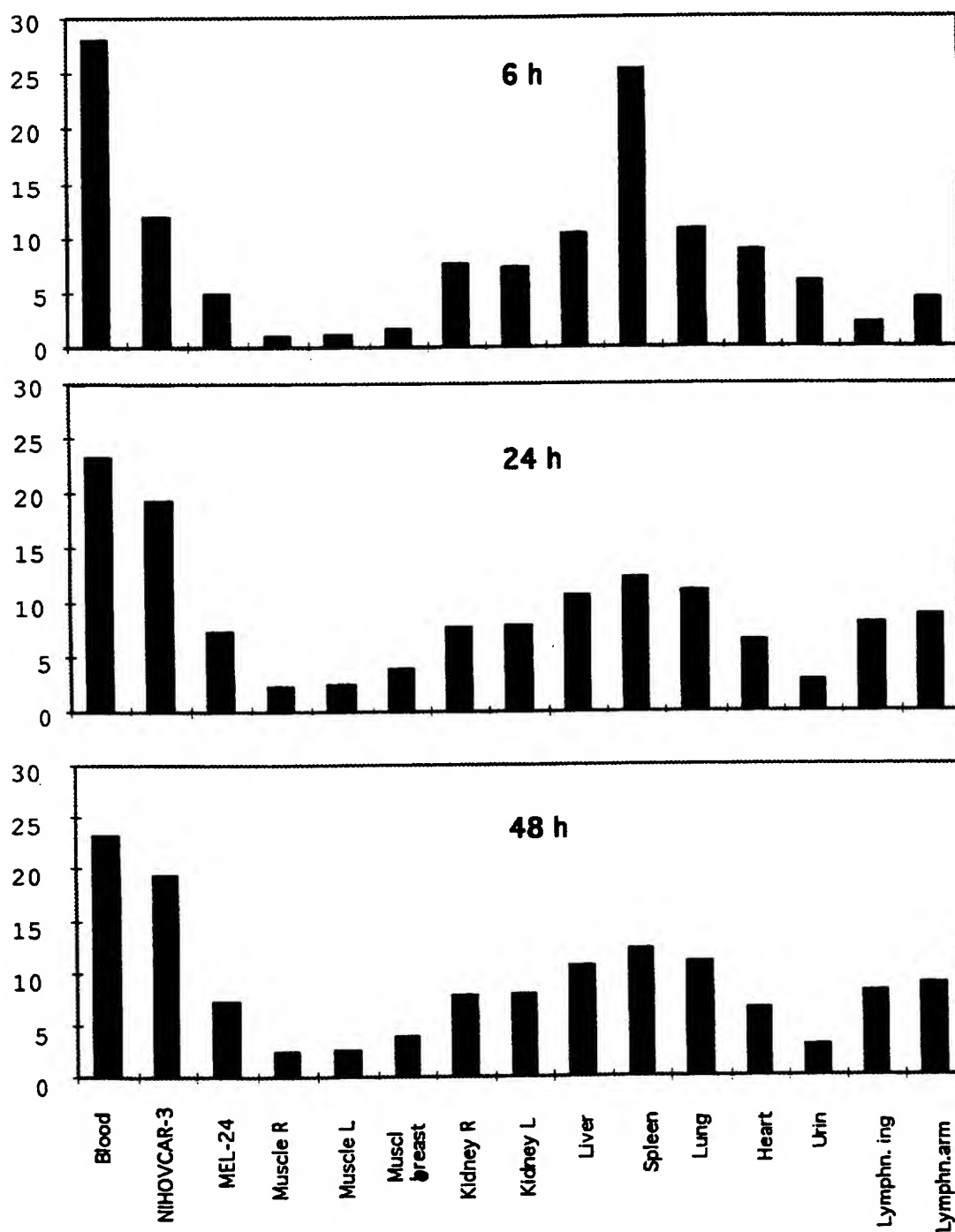
Figure 3

EXT1-7C12 uptake (%/ g)

Figure 4a

EXT1-7C12 uptake (%/ g)
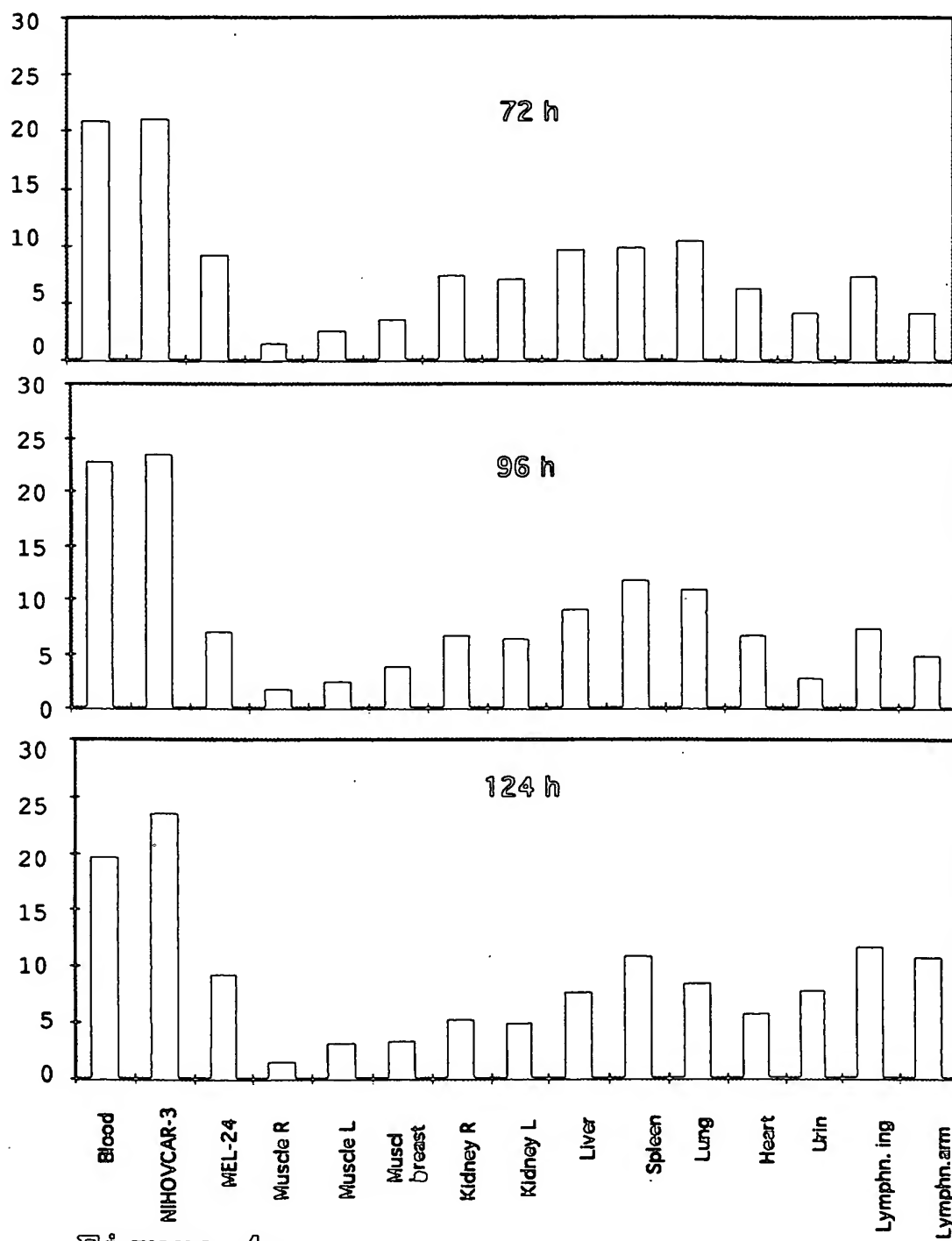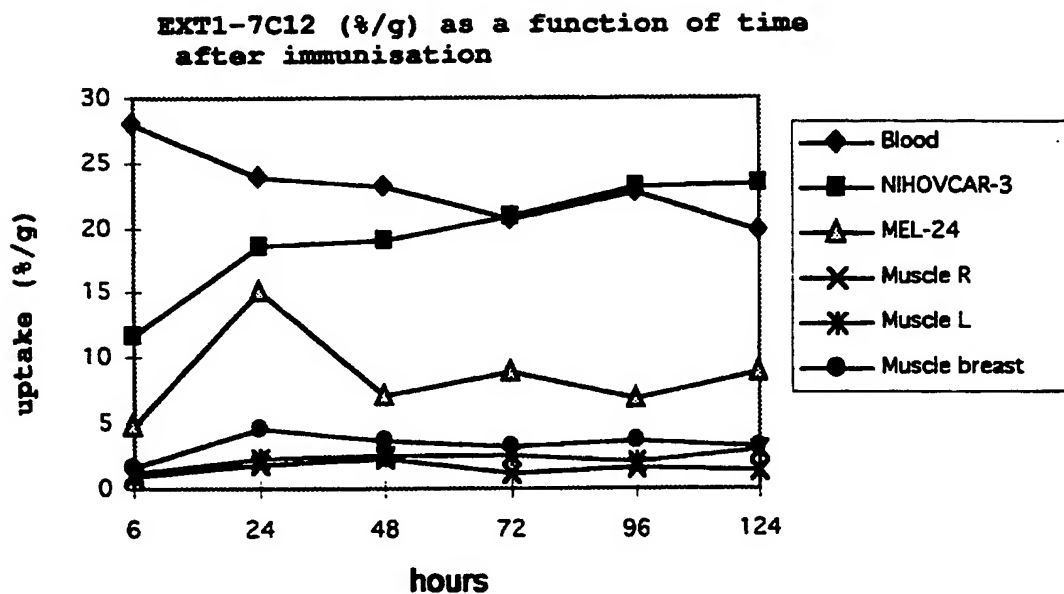
Figure 4a

Figure 4b.

ELISA of affinity purified antigen

Figure 5a

Figure 5b

a)

b)
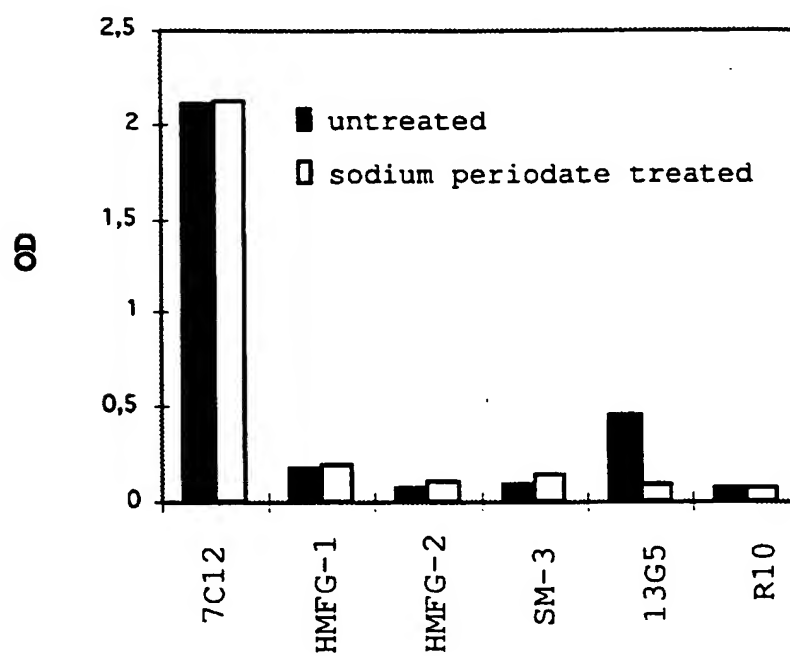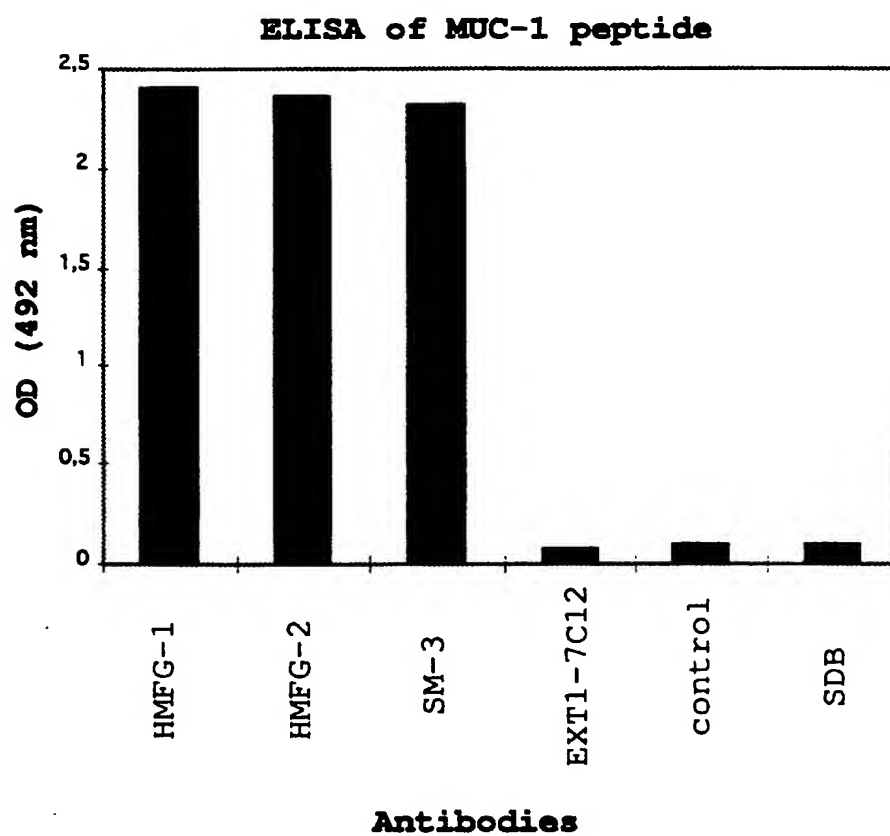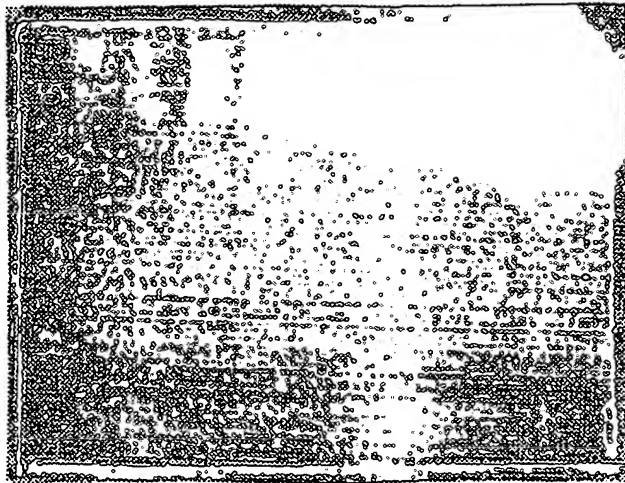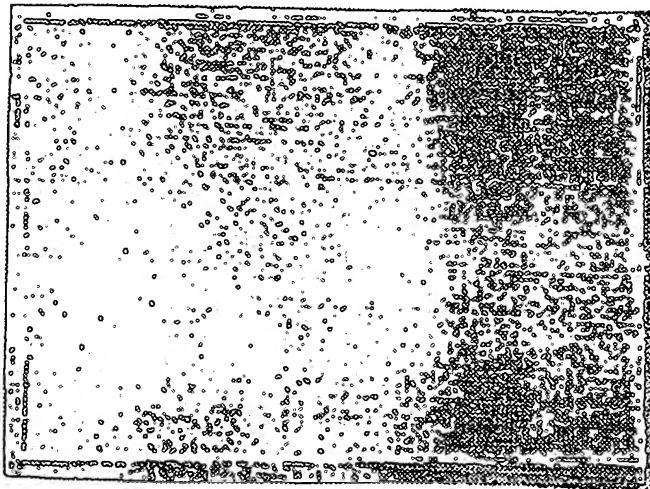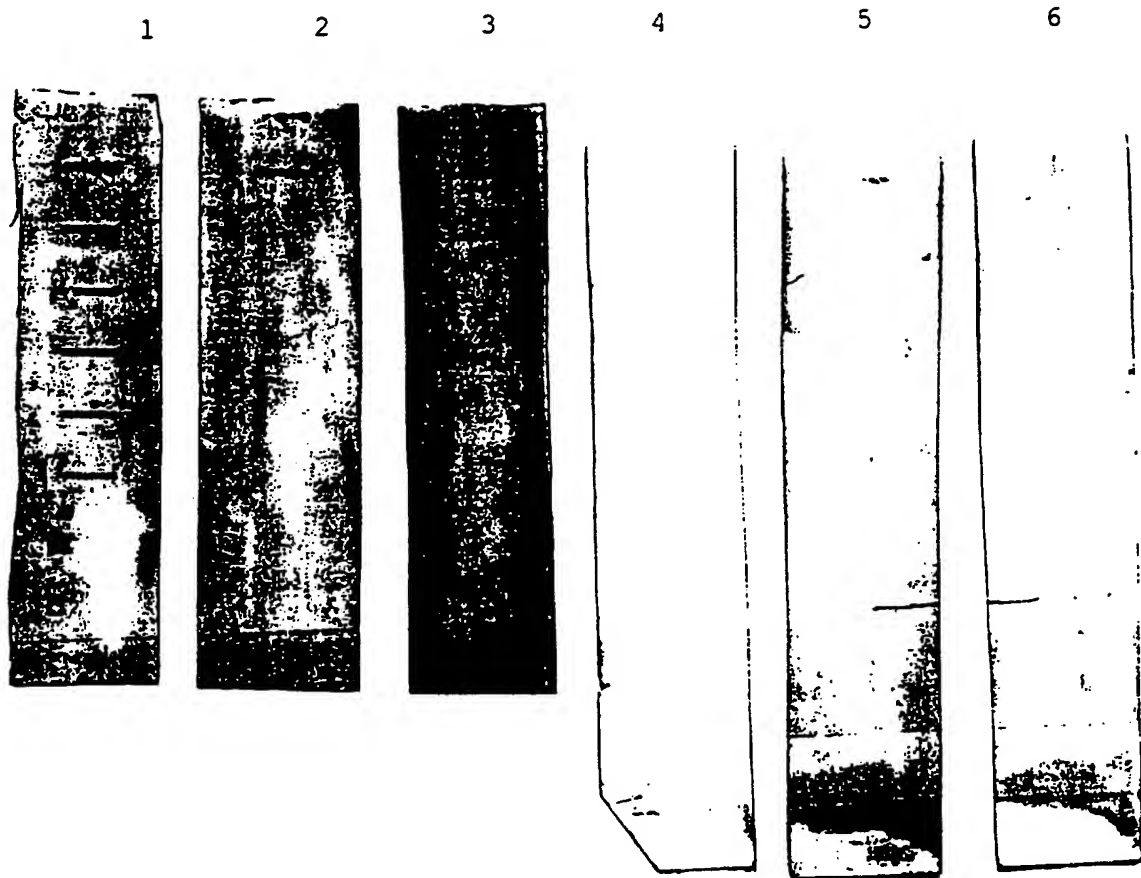
Figure 6

Figure 7

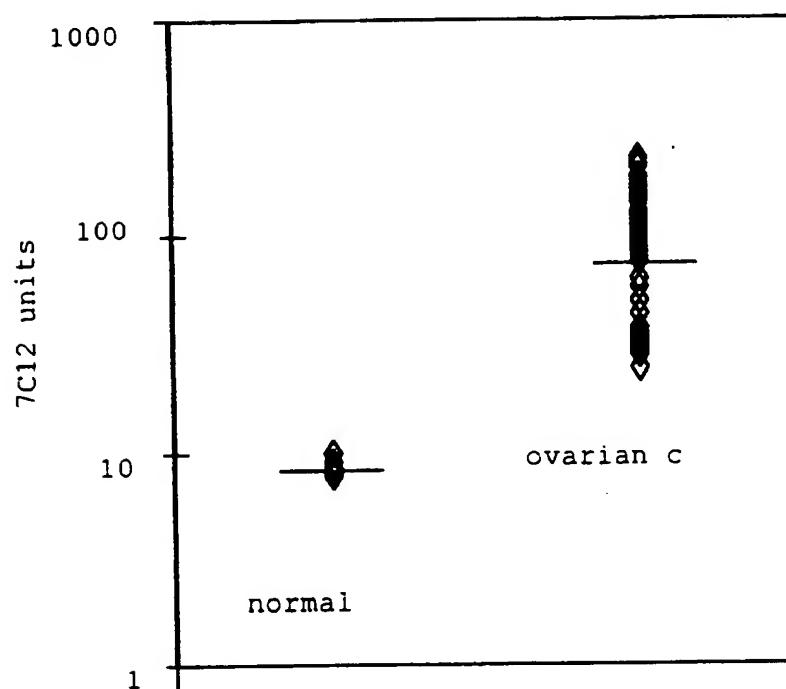7C12 antigen in patient sera

Figure 8

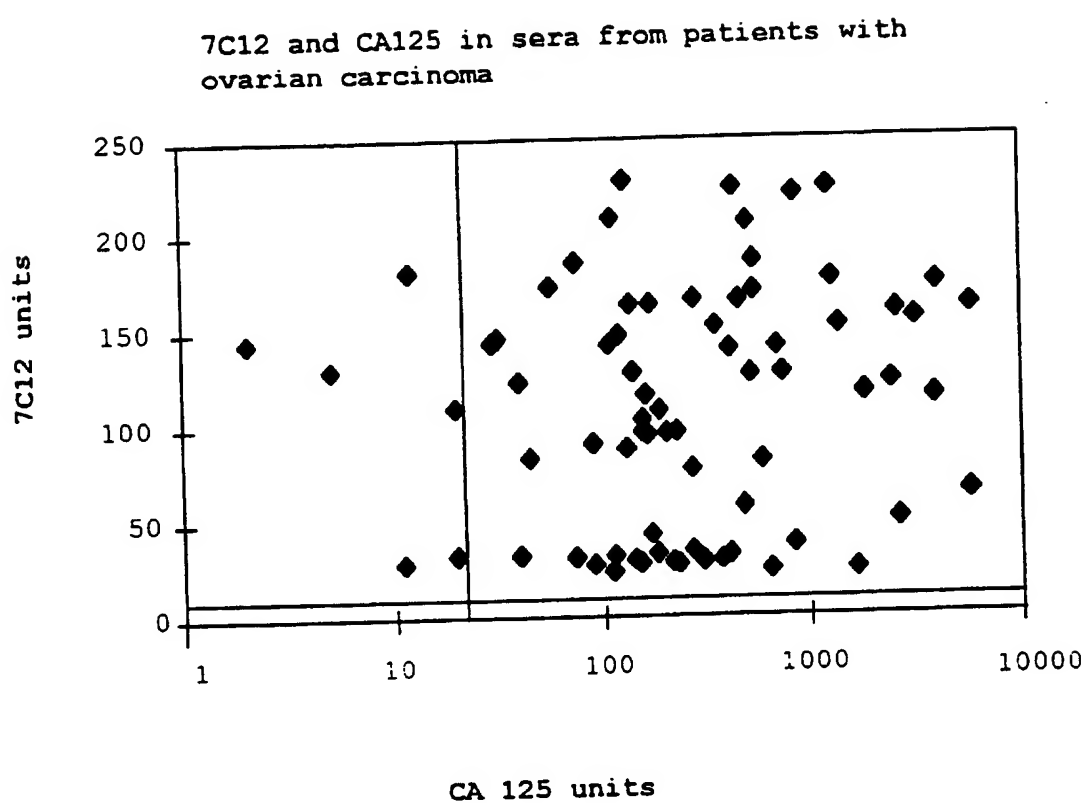7C12 and CA125 in sera from patients with
ovarian carcinoma

Figure 9

Interntional Application No

PCT/GB 96/00783

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   C07K16/30   A61K47/48   A61K49/00   A61K39/395   G01N33/577

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   C07K   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Section Ch, Week 8942 Derwent Publications Ltd., London, GB; Class B04, AN 89-303484 XP002009800 & JP,A,01 211 497 (AICHI-KEN) , 24 August 1989 see abstract ---	1,3,4,14
X	WO,A,92 07081 (US ARMY) 30 April 1992 see the whole document ---	1,3,4, 6-9,13
X	PATENT ABSTRACTS OF JAPAN vol. 018, no. 351 (C-1220), 4 July 1994 & JP,A,06 090785 (AICHI PREF GOV), 5 April 1994, see abstract ---	1,3,4, 7-9,14

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[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category°	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 226 419 (CETUS CORP ;US HEALTH (US)) 24 June 1987 see the whole document ---	10,11
A	WO,A,93 02358 (UNIV DUKE) 4 February 1993 see the whole document ---	14
A	DATABASE WPI Section Ch, Week 9416 Derwent Publications Ltd., London, GB; Class B04, AN 94-131287 XP002009801 & JP,A,06 078 787 , 22 March 1994 see abstract -----	

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		CA-A-	2093928	13-04-92
		EP-A-	0554356	11-08-93
		JP-T-	6506824	04-08-94
		US-A-	5525337	11-06-96
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		EP-A-	0598000	25-05-94
		JP-T-	6509641	27-10-94
		US-A-	5486456	23-01-96

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